



Further, the DNA is useful for production of the above described enzyme, or serves as a therapeutic tool, diagnostic tool or research tool for the treatment of diseases caused by the abnormal expression of Gb3/CD77, or it may be useful for the treatment or diagnosis of diseases involved in the action of verotoxins.

[0090]

[Sequence Listing]

SEQUENCE LISTING

<110> Seikagaku Corporation

Koichi Furukawa

<120>  $\alpha$ 1,4-GALACTOSYLTRANSFERASE AND DNA ENCODING THEREOF

<130> P-7040

<140>

<141> 2000-02-14

<160> 2

<170> PatentIn Ver. 2.0

[0091]

<210> 1

<211> 1975

<212> DNA



- 42 -

<213> Homo sapiens

<220>

<221> CDS

<222> (134)..(1192)

<400> 1

aaggteggt gctgagccag ggcgtgtctc ccggaggcct gtgggctgcc aggatcccca 60  
cctctctgca atgggctgcc caggctgacc agccgggttcc tgetggaage tcttggtctg 120  
atctggggat acc atg tcc aag ccc ccc gac ctc ctg ctg cgg ctg ctc 169

Met Ser Lys Pro Pro Asp Leu Leu Leu Arg Leu Leu

1

5

10

cgg ggc gcc cca agg cag cgg gtc tgc acc ctg ttc atc atc ggc ttc 217  
Arg Gly Ala Pro Arg Gln Arg Val Cys Thr Leu Phe Ile Ile Gly Phe

15

20

25

aag ttc acg ttt ttc gtc tcc atc atg atc tac tgg cac gtt gtg gga 265  
Lys Phe Thr Phe Phe Val Ser Ile Met Ile Tyr Trp His Val Val Gly

30

35

40

gag ccc aag gag aaa ggg cag ctc tat aac ctg cca gca gag atc ccc 313  
Glu Pro Lys Glu Lys Gly Gln Leu Tyr Asn Leu Pro Ala Glu Ile Pro

45

50

55

60

tgc ccc acc ttg aca ccc ccc acc cca ccc tcc cac ggc ccc act cca 361  
Cys Pro Thr Leu Thr Pro Pro Thr Pro Pro Ser His Gly Pro Thr Pro

65

70

75

ggc aac atc ttc ttc ctg gag act tca gac cgg acc aac ccc aac ttc 409

Gly Asn Ile Phe Phe Leu Glu Thr Ser Asp Arg Thr Asn Pro Asn Phe  
80 85 90  
ctg ttc atg tgc tcg gtg gag tcg gcc gcc aga act cac ccc gaa tcc 457  
Leu Phe Met Cys Ser Val Glu Ser Ala Ala Arg Thr His Pro Glu Ser  
95 100 105  
cac gtg ctg gtc ctg atg aaa ggg ctt ccg ggt ggc aac gcc tct ctg 505  
His Val Leu Val Leu Met Lys Gly Leu Pro Gly Gly Asn Ala Ser Leu  
110 115 120  
ccc cgg cac ctg ggc atc tca ctt ctg agc tgc ttc ccg aat gtc cag 553  
Pro Arg His Leu Gly Ile Ser Leu Leu Ser Cys Phe Pro Asn Val Gln  
125 130 135 140  
atg ctc ccg ctg gac ctg cgg gag ctg ttc cgg gac aca ccc ctg gcc 601  
Met Leu Pro Leu Asp Leu Arg Glu Leu Phe Arg Asp Thr Pro Leu Ala  
145 150 155  
gac tgg tac gcg gcc gtg cag ggg cgc tgg gag ccc tac ctg ctg ccc 649  
Asp Trp Tyr Ala Ala Val Gln Gly Arg Trp Glu Pro Tyr Leu Leu Pro  
160 165 170  
gtg ctc tcc gac gcc tcc agg atc gca ctc atg tgg aag ttc ggc ggc 697  
Val Leu Ser Asp Ala Ser Arg Ile Ala Leu Met Trp Lys Phe Gly Gly  
175 180 185  
atc tac ctg gac acg gac ttc att gtt ctc aag aac ctg cgg aac ctg 745  
Ile Tyr Leu Asp Thr Asp Phe Ile Val Leu Lys Asn Leu Arg Asn Leu  
190 195 200  
acc aac gtg ctg ggc acc cag tcc cgc tac gtc ctc aac ggc gcg ttc 793  
Thr Asn Val Leu Gly Thr Gln Ser Arg Tyr Val Leu Asn Gly Ala Phe

205                      210                      215                      220  
ctg gcc ttc gag cgc cgg cac gag ttc atg gcg ctg tgc atg cgg gac 841  
Leu Ala Phe Glu Arg Arg His Glu Phe Met Ala Leu Cys Met Arg Asp  
                    225                      230                      235  
ttc gtg gac cac tac aac ggc tgg atc tgg ggt cac cag ggc ccg cag 889  
Phe Val Asp His Tyr Asn Gly Trp Ile Trp Gly His Gln Gly Pro Gln  
                    240                      245                      250  
ctg ctc acg cgg gtc ttc aag aag tgg tgt tcc atc cgc agc ctg gcc 937  
Leu Leu Thr Arg Val Phe Lys Lys Trp Cys Ser Ile Arg Ser Leu Ala  
                    255                      260                      265  
gag agc cgc gcc tgc cgc ggc gtc acc acc ctg ccc cct gag gcc ttc 985  
Glu Ser Arg Ala Cys Arg Gly Val Thr Thr Leu Pro Pro Glu Ala Phe  
                    270                      275                      280  
tac ccc atc ccc tgg cag gac tgg aag aag tac ttt gag gac atc aac 1033  
Tyr Pro Ile Pro Trp Gln Asp Trp Lys Lys Tyr Phe Glu Asp Ile Asn  
285                      290                      295                      300  
ccg gag gag ctg ccg cgg ctg ctc agt gcc acc tat gct gtc cac gtg 1081  
Pro Glu Glu Leu Pro Arg Leu Leu Ser Ala Thr Tyr Ala Val His Val  
                    305                      310                      315  
tgg aac aag aag agc cag ggc acg cgg ttc gag gcc acg tcc agg gca 1129  
Trp Asn Lys Lys Ser Gln Gly Thr Arg Phe Glu Ala Thr Ser Arg Ala  
                    320                      325                      330  
ctg ctg gcc cag ctg cat gcc cgc tac tgc ccc acg acg cac gag gcc 1177  
Leu Leu Ala Gln Leu His Ala Arg Tyr Cys Pro Thr Thr His Glu Ala  
                    335                      340                      345

atg aaa atg tac ttg tgagggggccc gccaggtcac ctccccaacc tgctcctgat 1232

Met Lys Met Tyr Leu

350

ggggcactgg gccgcccttc ccggggaggc aagattgagg gcccgggaga gggaggcccg 1292

agctgccacc gggcttaggc aggctgttga ggagctgtgg gagcaggccc agtgggaggc 1352

tgtggacacc ccgaggacag tgtcctgtct cgaggcaggg ctgacacatg gtgccatagc 1412

cagcggaggg cgctcagtga gtgccccggg ccttctagac aacaggcagg aaggatgaac 1472

ctcagggcac cccaggtgg tgccgaaagc caggcagttg ggacagaggt gcccacgagg 1532

gcagaggccg gtgctaaggg gatggggaag aagggacaag attcccagag aggagaggag 1592

gctgttggtg ggaaagtggc agggctgggg gagaccagc cccaagggtc cggggcggag 1652

gatgctttgt tcttttctgg ttttggttcc tctttcggg ggggtggggg aggtcaacag 1712

ggactgagtg gggcagaggc ccagaagtgc cagcctgggg agccgtttgg gggcagcccc 1772

ttctgcccac cccatccttc ttctctcca gagatgccag gggggcgtgt atgctctgcc 1832

ccttccctca gacaggggct ggggtggggg gctcttttagg ctcaggagaa gcattttaaa 1892

gaaaccccca ccctgccgcc cgcattataa acacaggaga ataataata gaataaaaagt 1952

gaccgactgt caaaaaaaaaaaa aaa 1975

<210> 2

<211> 353

<212> PRT

<213> Homo sapiens

<400> 2

Met Ser Lys Pro Pro Asp Leu Leu Leu Arg Leu Leu Arg Gly Ala Pro

1

5

10

15

Arg Gln Arg Val Cys Thr Leu Phe Ile Ile Gly Phe Lys Phe Thr Phe  
20 25 30  
Phe Val Ser Ile Met Ile Tyr Trp His Val Val Gly Glu Pro Lys Glu  
35 40 45  
Lys Gly Gln Leu Tyr Asn Leu Pro Ala Glu Ile Pro Cys Pro Thr Leu  
50 55 60  
Thr Pro Pro Thr Pro Pro Ser His Gly Pro Thr Pro Gly Asn Ile Phe  
65 70 75 80  
Phe Leu Glu Thr Ser Asp Arg Thr Asn Pro Asn Phe Leu Phe Met Cys  
85 90 95  
Ser Val Glu Ser Ala Ala Arg Thr His Pro Glu Ser His Val Leu Val  
100 105 110  
Leu Met Lys Gly Leu Pro Gly Gly Asn Ala Ser Leu Pro Arg His Leu  
115 120 125  
Gly Ile Ser Leu Leu Ser Cys Phe Pro Asn Val Gln Met Leu Pro Leu  
130 135 140  
Asp Leu Arg Glu Leu Phe Arg Asp Thr Pro Leu Ala Asp Trp Tyr Ala  
145 150 155 160  
Ala Val Gln Gly Arg Trp Glu Pro Tyr Leu Leu Pro Val Leu Ser Asp  
165 170 175  
Ala Ser Arg Ile Ala Leu Met Trp Lys Phe Gly Gly Ile Tyr Leu Asp  
180 185 190  
Thr Asp Phe Ile Val Leu Lys Asn Leu Arg Asn Leu Thr Asn Val Leu  
195 200 205  
Gly Thr Gln Ser Arg Tyr Val Leu Asn Gly Ala Phe Leu Ala Phe Glu

210	215	220	
Arg Arg His Glu Phe Met Ala Leu Cys Met Arg Asp Phe Val Asp His			
225	230	235	240
Tyr Asn Gly Trp Ile Trp Gly His Gln Gly Pro Gln Leu Leu Thr Arg			
	245	250	255
Val Phe Lys Lys Trp Cys Ser Ile Arg Ser Leu Ala Glu Ser Arg Ala			
	260	265	270
Cys Arg Gly Val Thr Thr Leu Pro Pro Glu Ala Phe Tyr Pro Ile Pro			
	275	280	285
Trp Gln Asp Trp Lys Lys Tyr Phe Glu Asp Ile Asn Pro Glu Glu Leu			
	290	295	300
Pro Arg Leu Leu Ser Ala Thr Tyr Ala Val His Val Trp Asn Lys Lys			
	305	310	315
Ser Gln Gly Thr Arg Phe Glu Ala Thr Ser Arg Ala Leu Leu Ala Gln			
	325	330	335
Leu His Ala Arg Tyr Cys Pro Thr Thr His Glu Ala Met Lys Met Tyr			
	340	345	350
Leu			

[Brief Description of Drawings]

[Fig. 1] It shows flow cytometry indicating the expression of Gb3/CD77 by L cells. The left diagram relates to L cells transfected with pCDM8 while the right diagram to L cells transfected with pVTR1/CDM8. The thick line indicates the result of cells stained with mAb38.13 and FITC-conjugated rabbit anti-rat IgG (secondary antibodies) while the thin line the result of cells stained only with

the secondary antibodies (control).

[Fig. 2] It shows TLC charts of glycolipids extracted from cells transiently transfected with  $\alpha 1,4$  Gal-T gene.

A: TLC of glycolipids extracted from L cells transfected with pCDM8 (VC) or pVTR1/CDM8 (TF). RBC represents neutral glycolipids extracted from human B red blood cells.

B: TLC immunostaining of Gb3/CD77 by mAb38.13.

[Fig. 3] It shows the hydropathy plot of a polypeptide of the present invention.

[Fig. 4] It shows the  $\alpha 1,4$  Gal-T activity in the extracts of transient transfectants of pVTR1.

A:  $\alpha 1,4$  Gal-T activity when LacCer was used as an acceptor.

B:  $\alpha 1,4$  Gal-T activity when various acceptors were used. PG represents paragloboside.

[Fig. 5] It shows the result of northern blotting of  $\alpha 1,4$  Gal-T gene.

A: the upper columns show the results of hybridization with a  $^{32}\text{P}$ -labeled probe derived from pVTR1, while the lower columns show the results of hybridization of the same membranes as in A with a  $\beta$ -actin cDNA probe(control).

B: the expression levels of mRNA of  $\alpha 1,4$  Gal-T gene were compared among various human tissues. The ordinate represents the percentage of the expression level of a given tissue with respect to the level of heart after correction with the control.

[Fig. 6] It shows flowcytometry of stable transfectant cells.



The left diagram relates to cells transfected with pSV2neo while the right diagram to cells transfected with pVTR1 and pSV2neo. The thin line indicates the number of cells stained with mAb38.13 and FITC-conjugated rabbit anti-rat IgG (secondary antibodies) while the thick line the number of cells stained only with the secondary antibodies (control).

[Fig. 7] It shows the results of MTT assay of L-neo and L-VTR1. The left graph shows the result of L-neo while the right one the result of L-VTR1.

[Fig. 8] It shows the effect of vero toxins on the cell growth.

[Fig. 9] It shows an electrophoresis indicating the result of DNA fragmentation assay.

[Document Name] Abstract

[Object] To provide  $\alpha$ 1,4-galactosyltransferase to transfer a galactose residue to C4 position of galactose residue of lactosylceramide or galactosylceramide, and DNA coding for the enzyme.

[Solving Means]

The following polypeptides (A) and (B), and DNAs encoding thereof:

(A) a polypeptide consisting of an amino acid sequence represented by the amino acid Nos. 46-353 in SEQ ID NO: 2; or

(B) a polypeptide which comprises an amino acid sequence including substitution, deletion, insertion or transposition of one or few amino acids in the amino acid sequence of (A) and which has an enzymatic activity to transfer a galactose residue from a galactose donor to C4 position of galactose residue of lactosylceramide or galactosylceramide which serves as an acceptor.

[Drawing Selected] Fig.1